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Insulin-like growth factor 2: A modulator of anti-apoptosis related genes (*HSP70*, *BCL2-L1*) in bovine preimplantation embryos

Mehdi Vafaye Valleh^{a,*}, Poul Hyttel^b, Mikkel Aabech Rasmussen^b, Lotte Strøbech^c

^a Department of Animal Science, Faculty of Agriculture, University of Zabol, Zabol, Iran

^b Department of Basic Animal and Veterinary Sciences, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark

^c EmbryoTrans Biotech ApS, Copenhagen, Denmark

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ABSTRACT

Intrinsic defects within the embryos, reflected by elevated cell death and low proliferative ability, are considered the most critical factors associated with bovine infertility. The identification of embryonic factors, which are responsible for successful embryo development, is thus critical in designing strategies for infertility intervention. In this experiment, the possible mechanisms involved in both blastomere proliferation and regulation of cell death were studied by analysis of relative expression patterns of *IGF-II*, *BCL2-L1*, *BAK1*, and *HSP70* in 3 classes of morphological quality groups (e.g., excellent, good, and poor) of bovine blastocysts produced by IVF. Variation in total blastocyst cell numbers as well as their allocation to inner cell mass and trophectoderm lineages were also determined by differential CDX2 staining. Results showed that transcript levels for *IGF-II*, *BCL2-L1*, and the *BCL2-L1/BAK1* ratio were higher in excellent- and good-quality blastocysts compared with low-quality blastocysts ($P < 0.01$), whereas mRNA levels for *HSP70* were higher in low-quality blastocyst compared with excellent-quality bovine blastocysts ($P < 0.05$). In addition, excellent-quality blastocysts displayed not only greater total cell number but also greater mean inner cell mass/total cell number proportion than that of poor-quality blastocysts ($P < 0.01$). The expression levels of *IGF-II* showed negative correlation with the levels of *HSP70* ($r = -0.70$; $P < 0.05$); however, the correlation of expression levels of *IGF-II* with both of *BCL2-L1* ($r = 0.91$; $P < 0.01$) and the ratio of *BCL2-L1/BAK1* ($r = 0.78$; $P < 0.05$) were highly positive. There was no correlation between the expression levels of *IGF-II* and *BAK1* genes. In conclusion, these observations suggested that levels of endogenous *IGF-II* transcripts might be associated with the quality of IVF embryos by regulating either apoptosis-related genes or mitogenic actions in bovine preimplantation embryos.

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1. Introduction

Embryonic death has a significant influence on reproductive efficiency in cattle [1]. There are likely to be

various reasons for embryonic death, but an intrinsic defect within the embryo seems to play an important role [2,3]. Although there is very little information on the intrinsic defects in the embryo, it has been shown that embryo quality determined by blastocyst total cell number, the ratio of inner cell mass (ICM) to trophectoderm (TE) cells, and levels of apoptosis would be the most important index of embryo survival and pregnancy rate

* Corresponding author. Tel.: +98 9358237550; fax: +98 542 2242501.
E-mail address: mehdi.valleh@uoz.ac.ir (M. Vafaye Valleh).

after embryo transfer [4]. Accordingly, decrease in blastocyst cell number and increase in blastocyst apoptotic index were evidenced in morphologically poor-quality blastocysts [5,6]. Studies in human, murine, and bovine species have shown that the blastocyst proliferative potential and blastocyst apoptotic cell index are regulated by “survival” factors produced both by the embryo itself and by the maternal reproductive tract [7,8]. Insulin-like growth factor-II (IGF-II) is one of the best characterized pro-mitogenic and anti-apoptotic molecule, which in the cow is secreted by the embryo [9,10] and reproductive tract tissues [11,12]. Although promitogenic and anti-apoptotic effects of IGF-II have been reported for the cleavage stages of preimplantation development in several mammalian species *in vitro* [8,13,14], the underlying mechanisms of these embryotrophic effects of IGF-II with respect to embryo quality remain unknown. Nevertheless, both of Bcl2 and heat shock protein 70 (HSP70) family members have been shown to be involved in regulation of apoptosis during early embryo development [15–17].

As molecular mechanisms responsible for intrinsic defects in the embryos are not yet completely understood, it was hypothesized that various morphological grades of blastocysts may express different levels of both IGF-II and apoptosis regulatory genes. Therefore, the objective of the present study was to test whether expression profiles of endogenous IGF-II are correlated with various morphological qualities of bovine blastocysts. To find more details on the underlying mechanisms of embryotrophic actions of IGF-II, we analyzed the correlation of expression patterns between *IGF-II* and *BCL2-L1* and *HSP70* and *BAK1* genes as well as the relationships of blastocyst cell number and cell allocation to the ICM and TE lineage with the levels of *IGF-II* expression. The expression of selected genes and blastocyst cell number and cell allocation are considered as reliable predictors of embryo developmental competence [6,18,19].

2. Materials and methods

Chemicals and reagents were obtained from Sigma-Aldrich Co. (St. Louis, MO) unless otherwise stated.

2.1. *In vitro* embryo production

2.1.1. Oocyte collection and IVM

Bovine ovaries were collected from a local slaughterhouse and then transported to the laboratory in 0.9% physiological saline solution at 29 to 32 °C in a thermo container. The immature cumulus-oocyte complexes (COCs) aspirated from antral follicles (2–8 mm in diameter) were washed 3 times in Hepes-buffered TCM-199 wash medium (M2520) supplemented with 50-μg gentamicin mL⁻¹ and 0.5 mg BSA mL⁻¹. The COCs were washed once after retrieval in IVM medium before maturation. Groups of 35 to 40 COCs with an evenly granulated cytoplasm and surrounded by more than 3 layers of cumulus cells were placed in 500-μl maturation medium in four-well dishes (Nunc, Roskilde, Denmark) and cultured without oil overlay for 24 hours at 38.8 ± 0.2 °C in 5% CO₂ in ambient humidified air. The IVM medium consisted of Dulbecco's

Modified Eagle Medium supplemented with 5% fetal calf serum (FCS, produced by the Danish Veterinary Institute, Copenhagen, Denmark), 2 IU/mL gonadotropins (2:1 mixture of PMSG:hCG; Suigonan, VetPharm, Løgstør, Denmark), 0.2-mM pyruvate (P3662), 50-ng epidermal growth factor (EGF; 4127), and 50 g/mL gentamicin.

2.1.2. Sperm preparation and IVF

Frozen-thawed semen from proven high fertile bulls, (Viking Genetics, Randers, Denmark) were thawed at 37 °C and washed twice by centrifugation at 328 × g for 5 minutes in noncapacitation medium. The mature oocytes in groups of 40 were transferred into four-well dishes containing 400 μl of fertilization medium (Fert-TALP) and inseminated with viable spermatozoa at the concentration of 2 × 10⁶ spermatozoa/mL. The spermatozoa were co-incubated with matured COCs in TALP medium for 22 to 24 hours without oil overlay at 38.8 ± 0.2 °C in 5% CO₂ in ambient humidified air. The TALP medium consisted of Tyrode medium with 25-mM sodium bicarbonate, 10-mM lactate, 6-mg/mL fatty acid-free BSA, 0.2-mM pyruvate, 50-μg/mL gentamicin, 30-μg/mL heparin and PHE (20-μM penicillamine, 10-μM hypotaurine, and 1-μM epinephrine).

2.1.3. *In vitro* culture and embryo collection

After 22 to 24 hours of IVF, the presumptive zygotes were liberated from surrounding cumulus cells and excess spermatozoa by vortexing for 3 minutes. After 3 times wash in Hepes-buffered tissue culture medium (TCM-199), cumulus-free zygotes were transferred in groups of 35 in 500-μl BA 1216 culture media (Origio, Maløv, Denmark) with an overlay of oil at 38.8 ± 0.2 °C in 5% O₂, 5% CO₂ for 168 hours. After culture period, the resulting blastocysts were morphologically classified into three grades (grade A, excellent; grade B, good; and grade C, poor) according to Avery et al. [20]. After morphological classification, all grade embryos were collected and frozen at –80 °C for subsequent mRNA expression analysis or were fixed in 4% paraformaldehyde and stored short term at 4 °C in PBS for differential cell staining.

2.2. Differential staining of embryos

Differential cell staining was carried out as described by Wydooghe et al. [21] with modification. Briefly, blastocysts were permeabilized with 1% Triton X-100 in PBS for 40 minutes, then DNA was denatured by exposure to 2N HCl for 20 minutes followed by 100-mM Tris-HCl (pH, 8.5) for 10 minutes at room temperature (RT). After denaturation, embryos were blocked in the Blocking Solution (2% BSA in the PBS) for 40 minutes at RT and then incubated with the primary CDX2 monoclonal (BioGenex, AM392-ready to use) for 1 hour at RT. After washing (3 times for 20 minutes in PBS at RT), the embryos were treated with secondary antibodies of Alexa Fluor 594-labeled Goat Anti-Mouse IgG (Molecular Probes, Leiden, The Netherlands) for 1 hour at RT. After another wash step (3 times 20 minutes), DNA was stained with Hoechst (1% Hoechst 33342 in PBS) for 2 minutes at RT. Blastocysts were mounted onto a clean glass slide with a drop of fluorescent Mounting Medium

(Dako, Glostrup, Denmark). The embryos were examined under a fluorescence microscope (Leica, Solms, Germany) equipped with a DFC 350 digital camera (Leica). The total number of cells per blastocyst and cell numbers in TE and ICM cells were counted. The experiments were replicated 3 times. In each replication, 4 to 7 embryos per group were processed.

2.3. Gene expression analysis

2.3.1. Primer design

Primer sequences for all target genes except *IGF-II* and *GAPDH* were obtained from previously published data in porcine and bovine. *IGF-II* and *GAPDH* primers were designed using Premier 6.0 software (Premier Biosoft International, Palo Alto, CA). Moreover, a Basic Local Alignment Search Tool search was conducted to confirm the specificity of the nominated primers (<http://www.ncbi.nlm.nih.gov/BLAST>). The target and reference primer sequences and their expected product sizes are displayed in Table 1.

2.3.2. RNA extraction and cDNA synthesis

Messenger RNA from pools of 5 to 7 morphologically poor blastocysts, 5 good blastocysts, or 5 excellent blastocysts was extracted using the NucleoSpin RNA XS Kit (Clontech, Mountain View, CA) according to the manufacturer's instructions. RNA samples were converted to cDNA with RevertAid first-strand cDNA synthesis kit (Fermentase, Glen Burnie, MD) using random hexamer primers. Samples were stored in the freezer (−20 °C) until analysis.

2.3.3. Quantitative real-time PCR (qPCR)

The mRNA levels were quantified using a LightCycler 480 SYBR Green I Master mix (Roche Applied Science) on a LightCycler 480 Instrument (Roche Diagnostics, Indianapolis, IN). The qPCR mixture consisted of 2-μl diluted cDNA (10 ng), 0.5-μl forward and reverse primers (10 mM), 5-μl SYBR Green I master mix, and 2-μl water to a total volume of 10 μl. Quantitative real-time PCR reactions were performed according to the following thermal cycling conditions: 95 °C for 10 seconds, followed by 45 to 55 PCR cycles of 95 °C for 10 seconds, annealing for 10 seconds at 58 to 60 °C, and extension for 20 seconds at 72 °C (Table 1).

The qPCR experiments were replicated with 3 separate pools of embryos for each quality grade, and a reaction without template served as negative control. To verify qPCR product identity, melting point curves were analyzed after amplification. The relative quantification of gene expression was analyzed by the comparative C_p method [26], using LightCycler 480 software (release 1.5.0 SP4). A standard curve was included on the assay plate for the relative quantification. To correct the differences in RNA quantities, relative gene expression levels were normalized using the geometric mean of three reference genes (Using Norm-Finder algorithm *GAPDH*, *B-actin*, and *H2A* were identified as the most stable housekeeping genes among the set of tested genes) according to Vandesompele et al. [27]. The PCR product sizes were confirmed by gel electrophoresis on a standard 2% agarose gel stained with ethidium bromide and visualized by exposure to ultraviolet light.

2.4. Statistical analysis

Differences in relative mRNA expression assayed by qPCR as well as embryo cell number and relative lineage size (ICM/TE) were tested for significance by analysis of variance (repeated measurement ANOVA) followed by Tukey–Kramer multiple comparisons, using the JMP statistical software version 7.0 (SAS Institute Inc., Cary, NC). Pearson pairwise correlation coefficient was used to determine the degree of correlation between expression patterns of selected genes in each groups of blastocyst quality. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1. Expression of *IGF-II* and apoptosis-related genes with respect to blastocysts quality

In this study, we analyzed the expression of *IGF-II* and apoptosis-related genes with respect to morphological quality of bovine blastocysts. The genes were selected on the basis of the results of our previous experiment (not published data) and reports from other research groups [18,22].

The expression of *IGF-II*, *BCL2-L1*, *BAK1*, and *HSP70* mRNAs was detected in all blastocyst quality groups (A, B, and C). The relative abundance of *IGF-II* was found to be

Table 1
Primer sequences and cycling conditions used for real-time PCR.

Gene	GenBank accession number	Primer sequence (5' → 3')	T_m (°C)	Product size (bp)	Reference
<i>HSP70</i>	AY149619.1	GTTCAGAGGAAGCACAGA GTTGATGCTCTTGTAGGT	60 °C	361	[22]
<i>BAK1</i>	NM_001077918.1	AGAACCTAGCAGCACCAT CGATCTTGGTGAAGTACTC	60 °C	150	[18]
<i>BCL2-L1</i>	NM_001077486.2	GAAACCCCTAGTGCCATCA GGGACGTGAGTCACTGAAT	60 °C	196	[23]
<i>H2A</i>	U62674	GTCGTGCAAGCAAGGAG GATCTCGGCCGTTAGGTACTC	58 °C	182	[24]
<i>B-actin</i>	AF191490	CGTGGGCCGCCCTAGGCACCA TTGGCCTTAGGGTTCAAGGGG	60 °C	244	[25]
<i>IGF-II</i>	X53553	GGCAAGTTCTTCCAATATGA TGGCAGAATTACGACACT	58 °C	239	This article
<i>GAPDH</i>	XM_001252511.4	CGGCATCGTGAAGGAC GCAGCACCAGTAGAAGCAG	60 °C	138	This article

significantly higher in morphologically excellent- (grade A) and good-quality (grade B) blastocyst than in poor-quality (grade C) blastocysts ($P < 0.01$; Fig. 1B). Relative abundance of the antiapoptotic *BCL2-L1* transcript was also significantly higher in excellent- (grade A) and good-quality (grade B) blastocysts compared with poor-quality ones (grade C; $P < 0.01$; Fig. 1D). In contrast, transcript levels of *HSP70*, an important mediator of responses to cellular stress, were significantly higher in poor-quality blastocysts than those in the excellent-quality blastocysts ($P < 0.01$; Fig. 1A). Although, in the case of *BAK1*, no significant difference was observed among the three experimental groups ($P > 0.05$; Fig. 1C), but the ratio of *BCL2-L1* to *BAK1* expression was significantly higher in excellent and good blastocysts compared with poor blastocysts ($P < 0.05$; Fig. 1E).

In addition, the correlation analysis results demonstrated that the levels of endogenous *IGF-II* transcripts not only positively correlated with the levels of *BCL2-L1* ($r = 0.91$; $P < 0.01$) and *BCL2-L1* to *BAK1* ratio ($r = 0.78$; $P < 0.05$) but also negatively correlated with levels of *HSP70* expression ($r = -0.70$; $P < 0.05$).

3.2. Differential cell staining

Different morphological grades of in vitro-produced blastocysts were subjected to differential cell staining for cell allocation analysis (Fig. 2). The mean of total cell number in good- and excellent-quality blastocysts was significantly greater than that of poor-quality blastocysts ($P < 0.01$; Fig. 3). Moreover, the number of ICM cells and the number of TE cells of excellent- and good-quality blastocysts were significantly greater than those of poor-quality blastocysts ($P < 0.01$; Fig. 3). However, there was no significant difference between excellent- and good-quality blastocysts in terms of ICM and TE cell number ($P > 0.05$; Fig. 3). Notably, most of the low-quality blastocysts showed a higher frequency of fragmented cells in ICM compared with the other groups, which could be associated with early pregnancy loss (Fig. 2C). The percentage of ICM cells (ICM%) was significantly greater in excellent-quality than that of poor-quality blastocyst ($P < 0.05$; Figs. 2, 3).

4. Discussion

Despite the subjectivity of the morphological evaluation, embryo quality is known to be associated with pregnancy outcomes [28]. Accordingly, decrease in blastocyst cell number and increase in blastocyst apoptotic index, which evidenced in morphologically poor-quality blastocysts, have been attributed to imbalance in gene expression [5,6].

In the present study, expression of *HSP70* varied among the three quality groups of blastocysts, with a higher level of expression in morphologically poor-quality bovine blastocysts compared with the other groups. *HSP70* ensures the survival of cells by regulation of several prosurvival signaling cascades, including those mediated by *Akt*, *JNK*, and *NF- κ B* [29,30]. Accordingly, *HSP70* induction has been proposed as a valuable biomarker of embryo stress [16,31]. The expression levels of *HSP70* have shown to be negatively correlated with embryo quality and viability; such that *in vitro*-derived blastocysts display a significantly lower expression of *HSP70*

than their *in vivo* counterparts [19,32,33]. Frozen-thawed bovine blastocysts, which exhibit a significantly higher apoptotic index than nonfrozen ones, were also shown to express significantly higher levels of *HSP70* transcripts than their nonfrozen counterpart [31].

Relative transcript abundance of the proapoptotic gene *BAK1* did not show significant differences among the different blastocyst quality groups. Nothing is known about the role of *BAK1* during early development of the preimplantation bovine embryos; however, *BAK1* has been shown to be involved in the regulation of apoptosis during early embryogenesis of the pig [34,35], human [36], and mouse [37], in a developmental stage-specific manner. Although elevated expression of *BAK1* has been shown to play a critical role in the induction of apoptosis by inducing permeabilization of the mitochondrial outer membrane and by forming complexes with the protective *BCL2-L1* proteins [38], the experimental evidence suggests that post-transcriptional and/or post-translational modification is also involved in regulation of *BAK1* activation [39,40]. Therefore, one potential implication of these findings could be related to species-specific differences in the mechanisms that modulate the effects of *BAK1* gene.

In our study, the antiapoptosis *BCL2-L1* gene transcript was significantly higher in excellent- and good-quality blastocysts compared with those of poor quality. Accordingly, several studies have shown an important role of *BCL2-L1* in the regulation of embryonic cell death in pig [18,34,35,41], mouse [42], human [43], and cattle [44–46]. In contrast, some studies have shown that the levels of *BCL2-L1* transcripts did not correlated with blastocyst quality [47,48]. Experimental sample size limitations and differences in methodology of gene expression analysis among studies may be the main reasons for this discrepancy.

In the present study, the ratio of *BCL2-L1/BAK1* expression, as an indirect index of cytochrome C release and apoptosis [38], was also significantly higher in excellent- and good-quality blastocysts than that in poor-quality blastocysts. It has been demonstrated that the inhibition of *BCL2-L1* and/or induction of *BAK1* expression would alter the ratio of *BCL2-L1/BAK1* expression in favor of increase in mitochondrial membrane permeability transition [49]. An increase in permeability of the outer mitochondrial membrane could lead to the release of cytochrome C from mitochondria into the cytosol, which once present in the cytoplasm activate the caspase cascade of cell degradation [50]. Therefore, upregulation of *BCL2-L1/BAK1* ratio in excellent- and good-quality blastocyst compared with poor-quality ones, suggests the presence of antiapoptotic mechanisms that modulate the intrinsic mitochondrial apoptotic pathway [18]. This result is consistent with the mitochondria-dependent death pathway model, in which the interactions between the pro- and anti-apoptotic members of *BCL-2* family determine the fate of a cell to live or demise [51].

Another differentially regulated gene was *IGF-II*, which was found to be significantly higher in morphologically excellent- and good-quality blastocysts compared with poor-quality blastocysts. Several studies have reported that *IGF-I* is not expressed during early embryonic development of cattle, indicating that the biological

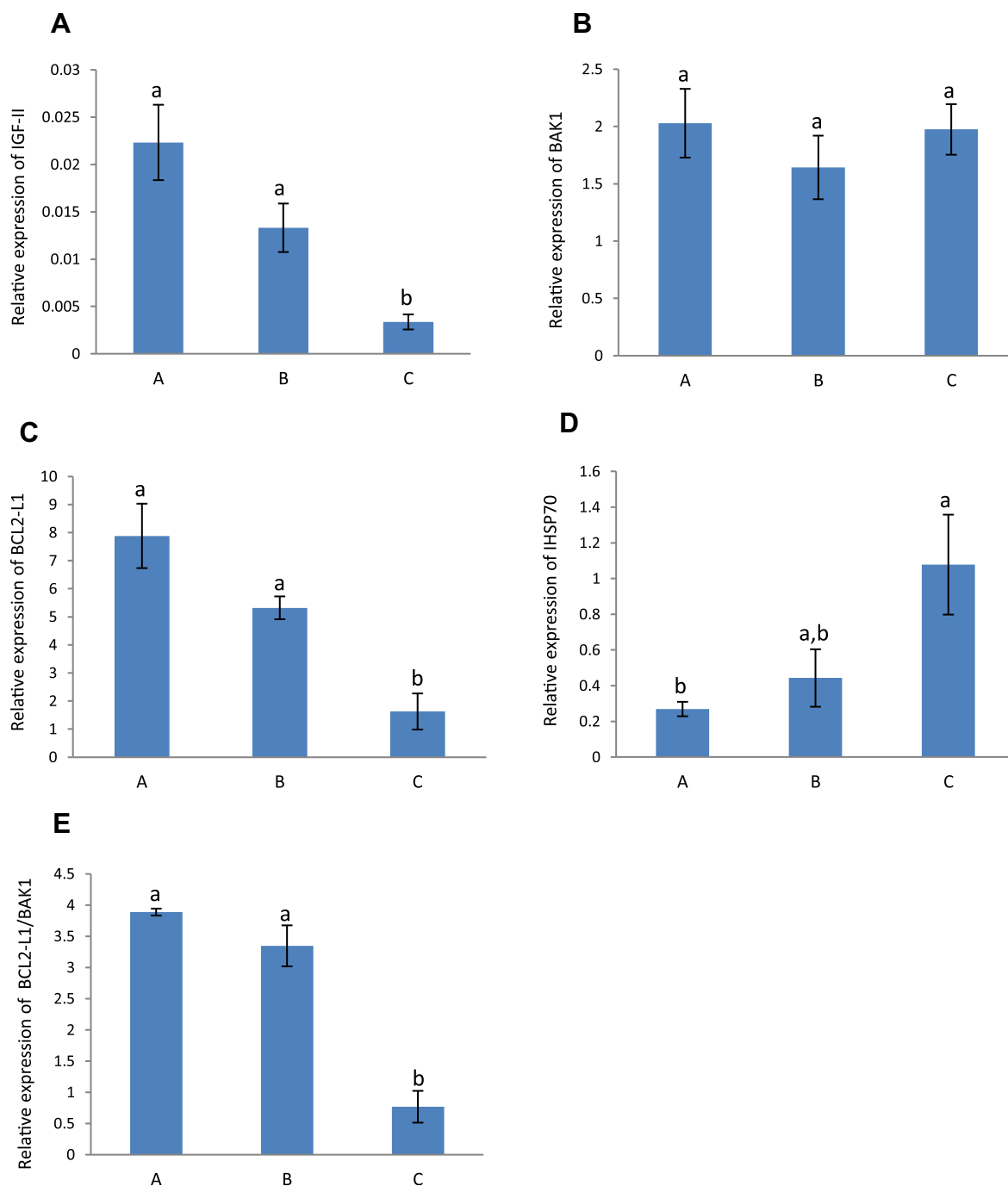


Fig. 1. Relative expression of *IGF-II*, *BAK1*, *BCL2-L1*, *HSP70*, and *BCL2-L1/BAK1* ratio, in three different morphological groups of bovine Day 8 blastocysts (A, B, and C represent excellent-, good-, and poor-quality *in vitro*-produced bovine blastocysts, respectively). Each value represents the mean \pm standard error of the mean of 3 samples. Values denoted by different superscripts differ significantly ($P < 0.05$) by Tukey test.

functions of IGFs might be performed by *IGF-II* [10,52]. It is demonstrated that *IGF-II* stimulates blastocyst formation, ICM mitogenesis, and protein synthesis in the pre-implantation embryo [52–54]. *IGF-II* also enhances the implantation competency of blastocysts possibly by the stimulation of production of embryonic Interferon-tau (IFN- τ) [55]. Embryonic IFN- τ plays a critical role in

establishment of pregnancy by inhibiting endometrial PGF2 α [56]. *IGF-II* was also found to be upregulated in embryos with high implantation potential compared with low implantation potential embryos [57]. Interestingly, it has also been reported that the levels of *IGF-II* expression are closely correlated with morphology of human embryos, so that embryos with high developmental potential

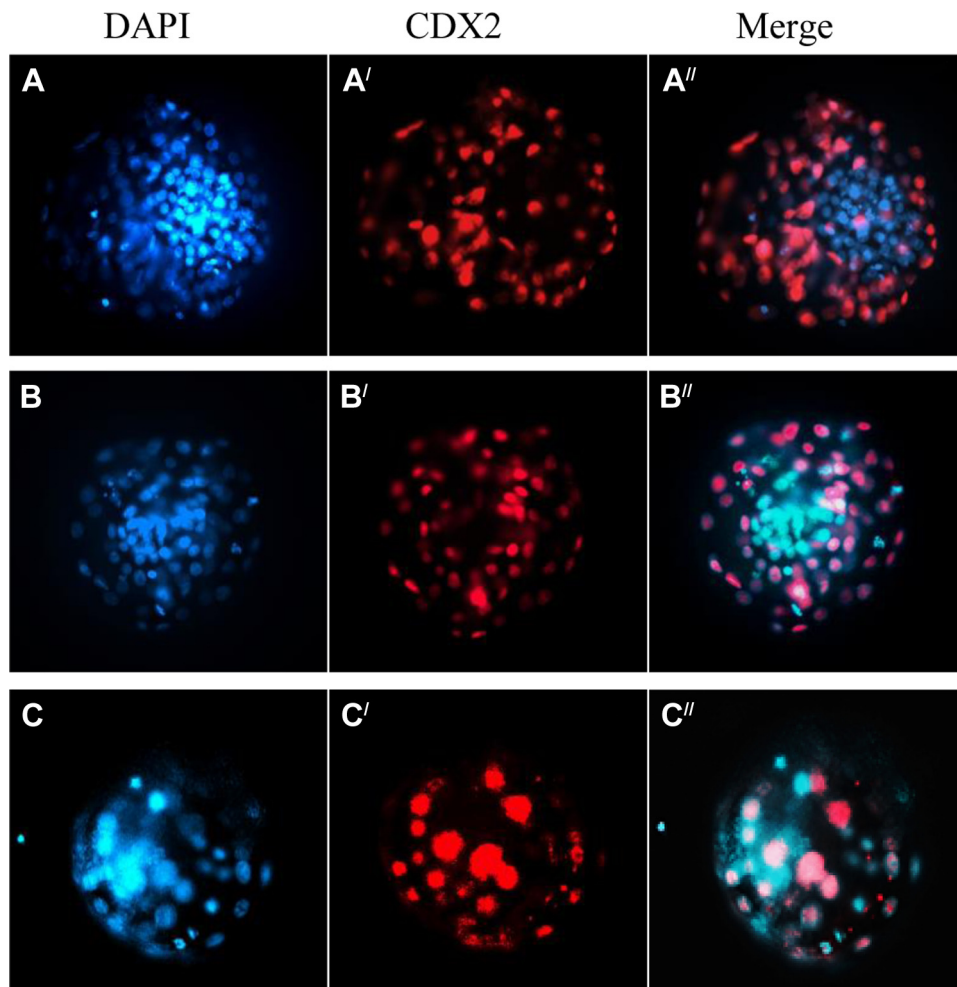


Fig. 2. Representative pictures of different quality bovine blastocysts stained with DAPI and CDX2, a marker of trophectoderm cells (A, B, and C represent excellent-, good-, and poor-quality *in vitro*-produced bovine blastocysts, respectively). The TE and ICM cell nuclei appeared in red and blue, respectively. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

may express higher levels of *IGF-II* compared with their lower competent counterpart [58]. In accordance, total blastocyst cell numbers and mean ICM proportion were also found to be greater in excellent- and good-quality blastocysts than those in poor quality; however, the mean ICM proportion only showed significant differences between excellent- and poor-quality blastocysts. There is good evidence that blastocyst proliferative potential and relative lineage sizes influence subsequent growth and viability of the embryo [4,28,59]. These findings are consistent with other reports indicating that the mean cell number and proportion of ICM of blastocyst recovered *in vivo* or *in vitro* decreased with reduction in embryo quality [60–62]. A growing body of evidence indicates that changes in transcript abundance at the blastocyst stage are frequently a direct consequence of perturbed transcription early in development [57,63]. Therefore, these findings may support the previous reports regarding the important role of endogenous *IGF-II* transcripts in controlling cell proliferation from the earliest stages of embryonic development [53,54].

The pairwise correlation coefficients between selected genes revealed that relative abundance of endogenous *IGF-II* not only positively correlated with *BCL2-L1* and the proportion of *BCL2-L1/BAK1* expression but also negatively correlated with *HSP70* expression, supporting previous findings regarding antiapoptotic actions of *IGF-II* during preimplantation development *in vitro* [13,14,64,65]. In our study, the *BCL2-L1/BAK1* ratio was mainly influenced by the levels of *BCL2-L1* transcripts rather than *BAK1* levels. Selective induction of *BCL2-L1* expression by *IGF-II* has also been reported in different cell lines [66,67]. Recent evidence suggests that activation of phosphatidylinositol 3-kinase-AKT/protein kinase B, which is activated on binding of *IGF-II* to its receptor [68], could in turn lead to activation of transcription of the *BCL2-L1* gene [67]. The upregulation of *BCL2-L1* could prevent the apoptosis by either binding to the pro-apoptotic *BAK1* [51] or closing the mitochondrial porin channel [69]. Similarly negative significant correlation between levels of *IGF-II* and *HSP70* transcripts may support the notion that the *IGF-II* reduces cellular stress and improves embryo viability [13,65]. However, there is

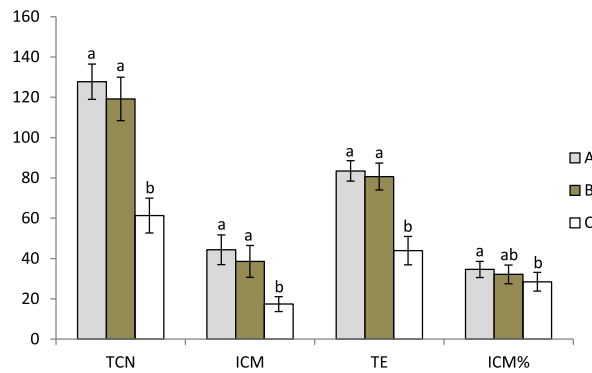


Fig. 3. The total cell number, inner cell mass (ICM), and trophectoderm cells and the ICM/total cell ratio (ICM%) in three different morphological groups of bovine blastocysts (A, B, and C represent excellent-, good-, and poor-quality *in vitro*-produced bovine blastocysts, respectively). The number of analyzed embryos (n) was as follows: 12 (A), 15 (B), and 10 (C). Data are presented as the mean \pm standard deviation. Different letters (a, b, and c) indicate significant differences between groups on the basis of one-way ANOVA followed by Tukey test ($P < 0.05$).

no evidence to elucidate the direct underlying molecular mechanisms linking *IGF-II* and *HSP70* transcripts levels. Nevertheless, both *IGF-II* and *HSP70* are involved in *p53*-mediated regulation of cell proliferation and apoptosis [70,71]. The *p53* is normally expressed at very low levels but may accumulate by post-transcriptional mechanisms in cells exposed to diverse forms of cellular stress, which leads to apoptosis and cell cycle arrest [72]. Induction of *HSP70* following diverse forms of cellular stress has been shown to be mediated by *p53* [73]. In contrast, *IGF-II* has been shown to downregulate *p53* and thus inhibit programmed cell death [74]. Therefore, *p53*-mediated regulation of cell death could be accounted for the negative correlation between *IGF-II* and *HSP70* expression that was found in the present study; however, further studies are needed to clarify the underlying mechanism.

4.1. Conclusions

We found that poor-quality embryos differ not only in their expression patterns of *IGF-II*, *BCL2-L1*, and *HSP70* but also in the number of ICM and TE cells when compared with their excellent counterparts. These results support the hypothesis that preimplantation embryos use the same mechanisms used in other cells to execute and regulate cell proliferation and death. Besides, results of correlation analyses suggest an important role of endogenous *IGF-II* signaling pathway in embryo survival and development. Expression analysis of these genes, therefore, potentially could be used as valuable biomarkers for selecting embryos with a higher potential of implantation or for evaluation and optimization of culture medium.

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